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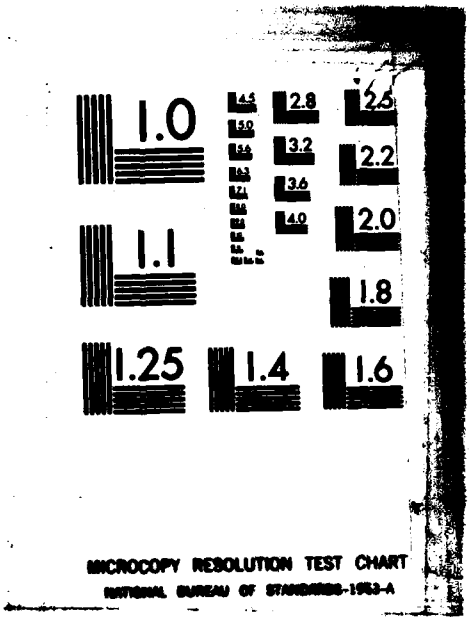
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COAGGLUTINATION REAGENT FOR THE RAPID PRESUMPTIVE IDENTIFICATION OF BACTEROIDES FRAGILIS

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COAGGLUTINATION REAGENT FOR THE RAPID PRESUMPTIVE IDENTIFICATION OF BACTEROIDES FRAGILIS

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Summary

A coagglutination test for presumptively identifying Bacteroides fragilis is described. The test utilizes protein A-containing staphylococci sensitized with speific antibody to rapidly identify suspensions of B. fragilis. Sensitization with 200 µl antiserum/ml 10% staphylococci produced a coagglutination reagent which exhibited specificity and sensitivity adequate for slide testing of colonies from primary cultures. The use of coagglutination for presumptive identification of bacteria is simple and reliable, requiring a minimum of training and equipment. Large numbers of colonies can be screened and subcultured for further testing if desired. Although coagglutination does not have the sensitivity of other test methods it provides results within 3 minutes.

INTRODUCTION

Infections due to nonsporeforming anaerobic bacteria are important causes of morbidity, particularly in post-surgical and traumatic injury patients (1,2). Since Bacteroides fragilis is involved in a significant percentage of anaerobic infections and frequently exhibits multiple antibiotic resistance (3,4,5), the rapid identification of this species is highly desirable thus aiding in the selection of appropriate antibiotic therapy.

Standard bacteriological identification of anaerobic bacteria may require several days. Immunofluorescence (6,7), radioimmunoassay (8), precipitin testing (9), selective media (10,11), gas chromatography (12) and susceptibility testing (13,14) have decreased the time required for presumptive identification, but have not simplified the procedures.

Coagglutination has been widely used to identify bacterial antigens (15), serological groups of bacteria (16) and to identify pathogenic bacteria directly from primary isolation plates (17). This technique is a simple, rapid and reliable procedure which is particularly well suited to small clinical laboratories or field use. The reagents for coagglutination are easily prepared by coating protein A-containing staphylococci with specific antibody. The test procedure is simple and identification can usually be accomplished within 3 minutes. This study demonstrates the use of coagglutination to provide rapid, presumptive identification of B. fragilis.

MATERIALS AND METHODS

Bacterial strains. The bacterial species used for antibody production and reagent testing were obtained from the American Type Culture Collection. These species were: Bacteroides fragilis (ATCC 23745), B. melaninogenicus (ATCC 25845), B. vulgatis (ATCC 8482), B. distasonis (ATCC 8503) and B. ovatus (ATCC 8483). In addition, two oral isolates of B. intermedius were included in the species specificity testing.

Coagglutination reagents. Antisera against B. fragilis were prepared by immunization of New Zealand white rabbits according to the method of Elhag and Tabaqchali (18). Antibody titers were determined by tube agglutination (19). Protein A-containing staphylococci (ATCC 12598) were harvested by the methods of Edwards et al (15,17) and reconstituted to a final concentration of 10% (vol/vol) in phosphate buffered saline (PBS) pH 7.4 with sodium azide (0.02%).

Serum from a single rabbit, R82-06 was used to prepare the coagglutination reagents. The optimum amount of antiserum for sensitizing the staphylococci was determined by dose-response titration with various amounts of antiserum against dilutions of antigen. The serum concentration which produced the reagent of greatest sensitivity was determined by testing different concentrations against serial two-fold dilutions of a suspension of B. fragilis adjusted to McFarland #4 (% absorbance) at 600 nm using a Bausch and Lomb Spectronic 21 spectrophotometer.

Specificity of the coagglutination reagents was determined by examining them for cross-reactivity with suspensions of other species of Bacteroides; B. ovatus, B. intermedius, B. vulgatis, B. distasonis and B. melaninogenicus, also adjusted to McFarland #4 in PBS.

Coagglutination testing was performed on bacterial suspensions by placing 10 µl bacterial suspension on a glass slide. The two suspensions were mixed with a wooden applicator stick and agitated for 3 minutes by rocking the slide. Slides were

examined for coagglutination at 1, 2 and 3 minutes. The reactions were graded as 3+: large aggregates within 1 minute, 2+:large aggregates within 2 minutes, 1+: fine aggregates visible with a hand lens or stereo microscope within 3 minutes, negative: no agglutination within 3 minutes.

Identification of bacterial colonies directly from primary plating media was accomplished by using a bacteriological loop to remove a single colony from agar surface and suspending it in a drop of PBS on a glass slide and then adding a drop of coagglutination reagent.

RESULTS

Antibody titers of sera from the immunized rabbits were from 1:32 to 1:64 by tube agglutination. Preimmunization sera did not contain antibody demonstrable by tube agglutination (Table I). Antiserum from a single rabbit, R82-06, was used to prepare the coagglutination reagents. The concentration of antiserum which produced the reagent of highest sensitivity without spontaneous agglutination was 400 µl antiserum/ml of 10% staphylococcal suspension (Table II). Unsensitized staphylococci and those sensitized with preimmunization serum did not coagglutinate B. fragilis suspensions. Subculture of suspensions on slides demonstrated the continued viability of B. fragilis after coagglutination.

TABLE I

Antibody titers of rabbits immunized with B. fragilis as determined by tube agglutination *

		Antiserum dilution						
Rabbit	Control**	1:4	1:8	1:16	1:32	1:64	1:128	Neg ***
R82-06	-	+	+	+	+	+	-	-
R81-90	-	+	+	+	+	-	-	-
R81-92	-	+	+	+	+	-	-	-

* As performed by Lambe & Moroz. 1976.

** Preimmunization serum

*** Normal saline

TABLE II

Comparison of sensitivity of coagglutination reagents prepared with varying levels of B. fragilis antiserum

Antiserum ul/ml	Numbers of B. fragilis cells per ml (X 10^6)					
	600	300	150	75	37.5	PBS
10	2+*	1+	-	-	-	-
25	2+	2+	±	-	-	-
50	3+	3+	2+	1+	-	-
100	3+	3+	3+	2+	-	-
200	3+	3+	3+	2+	±	-
300	3+	3+	3+	3+	3+	-
400	3+	3+	3+	3+	3+	-
500	3+	3+	3+	3+	3+	1+

* 3+ = large aggregates visible within 1 minute
2+ = large aggregates visible within 2 minutes
+ = fine aggregates visible with stereomicroscope at 3 minutes
- = no aggregates

With the testing methods used, reactions with other species of Bacteroides were not observed at sensitization levels of 200 µl antiserum/ml staphylococci. At 300 µl antiserum/ml, both oral isolates of B. intermedius and B. melaninogenicus formed fine aggregates with the reagent. When 400 µl serum was used to sensitize staphylococci, fine aggregates were observed with all of the Bacteroides suspensions (Table III).

TABLE III

Coagglutination of Bacteroides species by protein A containing staphylococci sensitized with different amounts of anti-B. fragilis antiserum

Bacteroides species	Antiserum amounts (µl/ml) 10	25	50	100	200	300	400	500	neg.**	Pre***
B. intermedius*	-	-	-	-	±	+	+	+	-	-
B. melaninogenicus	-	-	-	-	±	+	+	+	-	-
B. fragilis	+	+	+	+	+	+	+	+	-	-
B. ovatus	-	-	-	-	-	-	±	+	-	-
B. distasonis	-	-	-	-	-	-	±	+	-	-
B. vulgatis	-	-	-	-	-	-	±	+	-	-
saline	-	-	-	-	-	-	±	+	-	-

* Two strains

** Unsensitized staphylococci

*** Staphylococci sensitized with preimmunization serum

DISCUSSION

Rapid and accurate detection of B. fragilis provides very useful information to the clinician. A screening test which can provide presumptive identification of this anaerobe expedites the initiation of appropriate antibiotic therapy. Although several methods for rapid presumptive identification have been described, each has disadvantages. Fluorescent microscopy, radioimmunoassay and gas chromatography require sophisticated equipment and highly trained technicians. Identification through the use of bile and antibiotic discs requires 24-48 hours incubation beyond that required for primary isolation. The results of this study indicate that presumptive identification can be made within 1-3 minutes directly from primary isolation plates through use of coagglutination reagents. Since coagglutination reagents are prepared with non-viable staphylococci and testing does not kill the bacteria being tested, subculture directly from the test slide is possible if desired.

Although the sensitivity of the coagglutination reagents is far below that of immunofluorescence or radioimmunoassay, it is adequate for the examination of primary cultures and is far more suitable for field laboratory use. A wide variety of coagglutination reagents can be easily transported into the field and the species which

can be presumptively identified through the use of this type of reagent is limited only by the availability of specific antisera.

REFERENCES

1. Feller, J.M., and V. R. Dowell, Jr. 1971. "Bacteroides" bacteremia. Am. J. Med. 50:787-796.
2. Gorbach, S.L., and J. G. Bartlett. 1974. Medical progress: anaerobic infections. N. Eng. J. Med. 290:1237-1245.
3. Tally, F.P., J. G. Bartlett and S. L. Gorbach. 1978. Practical guide to anaerobic bacteriology. Lab. Med. 9:26-35.
4. Kislak, J.W. 1972. The susceptibility of Bacteroides fragilis to 24 antibiotics. J. Infect. Dis. 125:295-299.
5. Kirby, B.D., W.G. George, V.L. Sutter, D.M. Citron, and S.M. Finegold. 1980. Gram-negative anaerobic bacilli: their role in infection and patterns of susceptibility to antimicrobial agents. I. Little known Bacteroides species. Rev. Infect. Dis. 2:914-951.
6. Abshire, R.L., G.L. Lombard, and V.R. Dowell. 1977. Fluorescent antibody studies on selected strains of Bacteroides fragilis subspecies fragilis. J. Clin. Micro. 6:425-432.
7. Weissfeld, A.S. and A.C. Sonnenwirth. 1981. Rapid detection and identification of Bacteroides fragilis and Bacteroides melaninogenicus by immunofluorescence. J. Clin. Micro. 13:798-800.
8. Hoppes, W.L., J.P. Rissing, J.W. Smith, and A.C. White. 1980. Radioimmunoassay for Bacteroides fragilis infections. J. Clin. Micro. 12:205-207.
9. Rissing, J.P., J.G. Crowder, J.W. Smith, and A. White. 1974. Detection of Bacteroides fragilis infection by precipitin antibody. J. Infect. Dis. 130:70-73.
10. Lyznicki, J.M., E.L. Busch, and D. J. Blazevic. 1982. Medium for selective isolation and presumptive identification of the Bacteroides fragilis group. J. Clin. Micro. 15:123-129.
11. Chan, P.C.K. and R.K. Porschen. 1977. Evaluation of Kanamycinesculin bile agar for isolation and presumptive identification of Bacteroides fragilis group. J. Clin. Micro. 6:528-529.
12. Edson, R.S., J. E. Rosenblatt, J.W. Washington, II and J. B. Stewart. 1982. Gas-liquid chromatography of positive blood cultures for rapid presumptive diagnosis of anaerobic bacteremia. J. Clin. Micro. 15:1059-1061.

13. Draper, D.L. and A.L. Barry. 1977. Rapid identification of Bacteroides fragilis with bile and antibiotic disks. J. Clin. Micro. 5:439-443.

14. Yamazaki, E., K. Sugimoto, K. Niwano and J. Okada. 1982. A simple bile-disk method for the identification of Bacteroides fragilis. Microbiol. Immunol. 26:759-765.

17. Edwards, E.A. and R.L. Hilderbrand. 1976. A method for identifying Salmonella and Shigella directly from the primary isolation plate by coagglutination of protein A-containing staphylococci sensitized with specific antibody. J. Clin. Micro. 3:339-343.

18. Elhag, K.M., and S. Tabaqchali. 1978. A study of surface and somatic antigens of Bacteroides fragilis. J. Hyg. Comb. 80:439-449.

19. Lambe, D.W., Jr., and D. A. Moroz. 1976. Serogrouping of Bacteroides fragilis subsp. fragilis by the agglutination test. J. Clin. Micro. 3:586-592.

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